Sapropterin dihydrochloride treatment in Turkish hyperphenylalaninemic patients under age four

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Sapropterin enhances phenylalanine hydroxylase activity, thus lowering blood phenylalanine (Phe) concentration while increasing protein tolerance in sapropterin-responsive patients. Initiation of sapropterin treatment in responsive patients as early as possible, especially during the time when brain development is fastest, allows intake of more natural protein as well as micro- and macronutrients. Initiation of sapropterin treatment in the newborn period can make exclusive breastfeeding possible. Reports on the efficacy and safety of sapropterin in phenylketonuria (PKU) children under age four are limited in the literature. The purpose of this study is to evaluate the efficacy and safety of sapropterin treatment in infants and children with hyperphenylalaninemia (HPA) and to assess whether genotype analyses are of help in the prediction of responsiveness in these children. Clinical features as well as dietary characteristics were examined in 44 patients undergoing sapropterin treatment. Molecular genetic analysis was performed in 28 of these patients. Phe tolerance increased a median of 2.26-fold (0.88-4.23), from a median of 47.5 mg/kg/day to a median of 114 mg/kg/day ($p<0.001$). Phe levels could not be kept within normal limits in 5 patients, and thus treatment was stopped due to unsatisfactory metabolic control. In 9 patients, sapropterin treatment was started prior to the initiation of a Phe-restricted diet. Sapropterin treatment was found to be safe and efficacious in patients under age four. Although the BH_4 loading test and molecular genetic analysis proved to be useful in detecting responsive patients, these analyses did not enable us to make predictions as to long-term responsiveness.

Key words: phenylketonuria, sapropterin dihydrochloride, hyperphenylalaninemia, tetrahydrobiopterin, under age four.

There is accumulating evidence of the benefits of using sapropterin dihydrochloride in some patients with phenylalanine hydoxylase (PAH)-deficient phenylketonuria (PKU). Sapropterin, which is a synthetic analog of tetrahydrobiopterin (BH_4), enhances phenylalanine hydroxylase activity, thus lowering blood phenylalanine (Phe) concentrations while increasing protein tolerance[1]. Long-term beneficial effects of orally administered sapropterin in responsive PKU patients include improvement in metabolic control and increased Phe tolerance; in some patients such benefits lead to improvement in dietary adherence as well as in the quality of life[2]. Sapropterin improves tolerance of dietary Phe in about 20% of patients with PAH-deficient PKU[3].

No serious adverse events associated with the use of sapropterin have been encountered in the short term. Initiation of sapropterin treatment in responsive patients as early as possible, especially during the time when brain development is fastest, allows intake of more natural protein as well as micro- and macronutrients. Moreover, initiation of BH_4 treatment during the newborn period can make

exclusive breastfeeding possible. Therefore, it offers various potential advantages in young children in terms of growth, development and feeding preferences.

The use of sapropterin dihydrochloride has been recommended for the treatment of all BH_4-responsive PAH-deficient patients. However, its use in patients under age four has not yet been approved in many countries. Therefore, reports on the efficacy and safety of sapropterin in PKU children under age four are limited in the literature.

There is also no firm conclusion as to whether patients' genotypes can be of any help in predicting their responsiveness to BH_4[2,3]. The purpose of this study is to evaluate the efficacy and safety of sapropterin treatment in infants and children with hyperphenylalaninemia (HPA) and to determine whether genotype analyses can be of help in predicting responsiveness in these children.

BH_4 has been used in Turkey in patients under age four since 2011. In this retrospective analysis, we report our experience regarding BH_4 therapy and genotype analysis in PKU patients under age four.

Material and Methods

This was a single-center, cross-sectional study. HPA patients who were under age four at the time treatment was initiated were included in the study. All were diagnosed during routine neonatal screening. A 48-hour BH_4 loading test was used to detect responsive patients by administering a BH_4 dose of 20 mg/kg; a decrease >30% in blood Phe level was considered responsiveness. Sapropterin treatment was initiated as a dose of 20 mg/kg/day in all patients. Clinical features, as well as dietary characteristics in terms of Phe tolerance, presence of protein-substitute and low-protein foods, and plasma Phe levels during follow-up, were evaluated in 44 patients. Molecular genetic analyses were conducted in 28 of the patients. The possible presence of a BH_4 metabolism defect was excluded in all patients.

Dietary adjustment: Actual phenylalanine tolerance was calculated in each patient before the administration of sapropterin. Dietary phenylalanine intake was increased in increments of approximately 10 mg/kg on a weekly basis (max 300-350 mg/day) during the initiation and adjustment phase. Initially, vegetable protein (ordinary bread, pasta, rice, etc.) was given. Animal protein was introduced thereafter, and the amount of phenylalanine-free amino acid mixture was gradually decreased.

Molecular genetic analysis: All coding regions and exon-intron boundaries of the *PAH* gene were amplified. PCR fragments were purified with 96-well PCR filter plates (MinElute PCR purification kit, Qiagen Inc., Valencia, CA, USA), and mutation analysis was performed by direct sequencing of purified PCR products. Sequencing reactions were performed using the BigDye Terminator cycle sequencing kit (version 3.1) and analyzed on the ABI 3130 automated DNA sequencer (Applied Biosystems, CA, USA).

The Ethics Committee approved this study for Medical, Surgical and Drug Research at the Hacettepe University Faculty of Medicine, Ankara, Turkey (No: 40-861). Informed consent was obtained from subjects and their parents. All procedures followed were in accordance with the ethical standards of the responsible committees (institutional and national) on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

Statistical analyses

SPSS 11.5 for Windows was used for statistical analysis. The means, medians, percentages and standard deviations of the parameters were calculated. The normality of the distribution of the data was determined using the Kolmogorov–Smirnov test. The Mann–Whitney and Wilcoxon tests were used to determine whether there was a statistically significant difference between groups for nonparametric variables. Statistical significance was established as a p-value of <0.05.

Results

A total of 44 patients (28 boys, 16 girls) whose blood phenyalalanine levels were above 6.0 mg/dl were found to be sapropterin responsive. The median age at the time of starting BH_4 treatment was 1.9 years, ranging from 9 days to 3.8 years. The median treatment period was 26.7 months, with a range of 12 to 45 months. The current age range of the patients

Table I. Clinical Characteristics of the Patients

Number of patients	44 (16 female, 28 male)
Age at start of treatment (median)	1.9 years (9 days–3.8 years)
Current age (median)	3.8 years (1.3 years–6.9 years)
Treatment period (median)	26.7 months (12 months–45 months)

was 1.3–6.9 years (median 3.8 years) (Table I).

Dietary Adjustment

Dietary characteristics were evaluated in 44 patients who underwent sapropterin treatment. Among these patients, 39 were still on sapropterin treatment; sapropterin had been stopped in 5 patients due to unsatisfactory metabolic control. In 9 patients, sapropterin treatment had been started prior to the initiation of a Phe-restricted diet. Patients who had been on a Phe-restricted diet before the initiation of sapropterin treatment included 30 patients who were still on sapropterin treatment as well as the 5 patients in whom treatment had been discontinued.

Thirty patients were on a low-phenylalanine diet and twenty-four patients were using protein substitutes (61.5%) before sapropterin treatment was initiated. Sapropterin treatment was initated in 9 patients (9 days–1.14 years) with no history of Phe-restricted diet. Protein substitutes were stopped in 19 patients, with 5 patients (12.8%) remaining on the protein substitute while receiving sapropterin treatment. The median initial dose of protein substitute was 11.0 g of protein equivalent per day (PE/day) for 19 patients. The median intake of protein substitute did not change (pre-sapropterin=10.2 g PE/day, with sapropterin=10.2 g PE/day) for the 5 patients who required protein substitutes. Special low-protein foods were stopped in all patients.

The median blood phenylalanine concentrations (4.80 mg/dl) decreased after commencement of BH_4 treatment (to 3.75 mg/dl) in 39 patients, but the difference was not statistically significant (p=0.075). Phenylalanine tolerance increased by a median of 2.26-fold (0.88-4.23), from a median of 47.5 mg/kg/day to a median of 114 mg/kg/day (p<0.001).

In 5 patients, BH_4 did not improve metabolic control, as indicated by an increase in median blood phenylalanine levels (before sapropterin, median=3.71 mg/dl; with sapropterin, median=6.03 mg/dl; p=0.144). Phenylalanine tolerance also did not change significantly (going from a median of 50 mg/kg/day to a median of 58 mg/kg/day; p=0.225). All 5 of these patients were on a low-phenylalanine diet before BH_4 treatment.

BH_4 was started at 20 mg/kg/day in all patients. If the patient had satisfactory metabolic control, the total initial dosage was not changed, with the dose per kilogram decreasing as long as the child gained weight. Once the dietary phenylalanine introduction phase was completed, sapropterin was reduced to the dosage that was sufficient to maintain blood phenylalanine levels within normal limits (median=16.7 mg/kg/day).

During all follow-up visits, families were asked about any adverse effects. No side effects were noted in the patients treated with sapropterin.

Molecular Genetic Analysis

Mutation analysis for the *PAH* gene was performed in 28 patients. Allele frequencies are given in Table II. The mutations were found to be in the compound heterozygous state in 25 patients, while 3 patients were homozygous for the p.L48S, IVS10-7C>A and p.A104D mutations, respectively. All of the mutations found in this study were mutations known to cause phenylalanine hydroxylase deficiency.

Four different types of pathogenic mutations (3 splicing, 17 missense, 1 nonsense and 2 deletion) were detected in the patients. The most common mutated alleles in our cohort of PKU patients were p.E390G (14.3%), IVS10-11G>A (10.7%) and p.A300S (8.9%).

Discussion

Sapropterin treatment has been available as an important treatment option for responsive patients for nearly 15 years. Since approval by the European Medicines Agency (EMEA)

Table II. Frequencies of Alleles

Allele	Number	Percent
p.E390G	8	14.3
IVS10-11G>A	6	10.7
p.A300S	5	8.9
p.E178G	3	5.4
p.L15/L16fsdelCT	3	5.4
p.L48S	3	5.4
p.R261X	3	5.4
p.R408W	3	5.4
IVS10-7C>A	2	3.5
p.A104D	2	3.5
p.F382L	2	3.5
p.R408Q	2	3.5
p.T372S	2	3.5
IVS11+1G>C	1	1.8
p.Y198SfsX136	1	1.8
p.D296G	1	1.8
p.D394H	1	1.8
p.F121S	1	1.8
p.F55LfsX6	1	1.8
p.R241C	1	1.8
p.R241H	1	1.8
p.R243Q	1	1.8
p.R261Q	1	1.8
p.Y417C	1	1.8
not determined	1	1.8
Total	56	100

only applies to patients over age four, studies in patients who are younger than four have been limited. However, based on the limited number of studies reported in the literature, it appears that use of sapropterin does not cause any significant side effects[1]. Our study is one of those confirming the safety and effectiveness of BH_4 treatment in patients under age four .

Burton et al.[4] described patient selection, treatment administration, response evaluation and side effect management associated with sapropterin therapy in infants and children aged under four. Six case reports from four US metabolic clinics treating PKU with sapropterin in patients aged seven months to four years were presented. The study indicated that treatment with sapropterin is safe and generally well tolerated. Blood Phe levels were reduced, or, in patients with blood Phe that was well-controlled under a diet, maximum dietary Phe tolerance was increased. The authors suggested that sapropterin could be combined with a Phe-restricted diet for the purpose of controlling blood Phe levels in young patients responsive to sapropterin therapy[4]. In our study, 39 patients benefited from the treatment, and removal of protein substitutes from the diet was possible for 19 of the BH_4-treated patients. Although the diet could not be liberalized in 5 patients, who still required protein substitutes, it was possible to decrease the dosage of the substitute in 4 of these 5 children.

Leuret et al[2] reported the cases of 15 patients under age four who were receiving sapropterin therapy and demonstrated the safety and efficacy of this treatment for patients in

this age group. Long-term BH_4 therapy was initiated during the neonatal period (n=7) or at the age of 13 ± 12 months (n=8). The median duration of treatment was 23 months. BH_4 therapy significantly improved dietary phenylalanine tolerance (456 ± 181 vs. 1683 ± 627 mg/day, $p < 0.001$) and allowed a phenylalanine-free amino acid mixture to be discontinued or not even introduced in 14 patients. Additionally, in 8 patients who received the treatment after a few months of diet therapy, BH_4 treatment significantly decreased the mean phenylalaninemia, raised the percentage of phenylalaninemia tests within therapeutic targets and reduced the phenylalaninemia variance. No side effects were reported.

Neonatal screening and early treatment enabled the achievement of normal or near-normal IQ scores in appropriately treated children. It is clear from the existing literature, however, that apart from the active control of blood phenylalanine levels, there are other treatment-related factors affecting the outcome[5]. Couce et al.[6] reported the results of 16 patients undergoing long-term BH_4 treatment. Age at the start of treatment was under 4 years in 7 patients, 5 of whom had been receiving treatment since the first month of life. They concluded that testing at the time of diagnosis in the neonatal period is very appropriate, and if there is a positive response, the patient can be treated with BH_4 from the outset, with the advantage of being able to continue receiving breast milk.[6]

In our study, sapropterin treatment was started prior to the initiation of a Phe-restricted diet in 9 patients. Daily Phe tolerance increased by a median of 2.26-fold. Most of our patients benefited from BH_4 use, as evidenced by the liberalization of their diets to varying extents.

Sapropterin treatment, especially if initiated during the time when brain development is fastest, may allow for the intake of natural, high-quality protein as well as a micro- and macronutrient-rich diet, thus leading to better cognitive and executive function scores at older ages.

Treatment was initated at the maximum dose of 20 mg/kg/day in all responsive patients. If the patient had satisfactory metabolic control, the total initial dosage was not changed; thus, the per kilogram dosage decreased as long as the child gained weight. This could be a practical method for adjusting dosage, especially in the infantile period.

Patient genotypes might be of help in predicting responsiveness[7-9]. Leuders et al.[10] reported that *PAH* genotype might have an influence on sapropterin response in PKU. In their study, p.L48S/L48S and p.Y414C/p.Y414C were found always to be associated with response at a low dose of BH_4. The mutation p.Y414C (present on 16 alleles) was most frequently associated with response; patients with the mutation L48S on at least one allele always responded to sapropterin[10]. In the present study, molecular diagnosis confirmed PKU in 28 patients, in whom 55 alleles were found. Twenty-five patients were found to have a heterozygous mutation, while three patients were homozygous for p.L48S, IVS10-7C>A and p.A104D, respectively. The p.L48S, IVS10-7C>A, and p.A104D mutations are known to be sapropterin responsive.[8] However, in our one patient who was homozygous for the p.A104D mutation, BH_4 treatment was stopped due to uncontrolled Phe levels. Molecular genetic analysis was unavailable in two of our patients whose treatments were stopped. In the other two patients whose treatments were stopped, molecular genetic analyses were as follows: p.R243Q/p.R408Q and p.D296G/not detected. The most frequent mutations in the present study were p.E390G (14.3%), IVS10-11G>A (10.7%) and p.A300S (8.9%). Out of 25 responsive patients, 21 were found to have a responsive mutation on at least one allele. Some patients, especially those with a milder PKU phenotype, demonstrated a large and rapid decrease in blood phenylalanine, associated with a marked increase in their tolerance of dietary phenylalanine. As we performed molecular genetic analysis on responsive patients only, genotype-phenotype correlation was not possible, which was a limitation of our study.

No adverse effects were reported in our patient group. This was one of the first studies to investigate the administration of sapropterin treatment in a relatively large number of patients under age four. Our study confirms the safety and efficacy of sapropterin treatment in patients in this age group. Although the

BH_4 loading test and molecular genetic analysis proved to be useful in detecting responsive patients, these analyses did not enable us to make predictions as to long-term responsiveness.

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